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Direct Effects of H_2-Receptor Antagonists on Airway Smooth Muscle and on Responses Mediated by H_1- and H_2-Receptors

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Because it has been suggested that histamine H_2-receptor antagonists may worsen airway constriction in asthmatic patients, we investigated the comparative effects of three histamine H_2-receptor antagonists on guinea pig tracheal smooth muscle *in vitro*. When tested against resting tone, cimetidine, ranitidine and famotidine produced dose-related relaxation with pD_2 values (negative log of ED_{50} for relaxation) (±SE, n; eq 5) of 3.20 ± 0.04, 2.95 ± 0.16 and 2.97 ± 0.14, respectively. Concentrations that were below threshold for relaxation, did not elicit contraction. However, when the preparations were precontracted with histamine (10^{-5} M), dose-response curves for relaxation were shifted to the right, and low-concentrations of all three histamine H_2-antagonists augmented histamine-induced tone. When preparations were pretreated with cimetidine (10^{-6} to 10^{-4} M) and then tested for sensitivity to histamine, dose-response curves for histamine-induced contraction were shifted to the left (potentiated). These results provide further evidence for a modulatory effect of airway H_2-receptors on the contractile response to histamine. In addition, since the concentrations associated with potentiation of histamine-induced contraction were about the same for all three H_2-receptor antagonists ($\geq 10^{-5}$ M), our studies suggest a greater likelihood of airway constriction for the less potent H_2-receptor antagonists that must be administered in higher clinical doses. (Key words: Airway: guinea pig; smooth muscle; trachea. Pharmacology. Histamine H_2-receptor antagonists: cimetidine; famotidine; ranitidine.)

HISTAMINE H_2-RECEPTOR antagonists have been widely used by anesthesiologists both as a preanesthetic medication and in the treatment of critically ill patients in intensive care units. Nathan *et al.*,[1,2] however, found that H_2-receptor antagonists enhanced the bronchoconstrictor response to histamine challenge in humans and pointed out the possibility of increasing airway constriction in asthmatic patients. H_2-receptor antagonists also have been shown to potentiate the bronchoconstrictor response to histamine aerosols in a variety of experimental animals,[3–5] although the definite mechanism is unknown.

Because stimulation of H_2-receptors produces relaxation of airway smooth muscle in many species,[6,7] and because this action can partially antagonize the stimulant effect of histamine acting at H_1-receptors, administration of an H_2-receptor antagonist would be expected to potentiate the contractile response to histamine. Such potentiation has been demonstrated *in vitro* with human bronchial muscle[8] as well as in the tracheal smooth muscle of guinea pigs,[9,10] which is the preparation employed in the present study.

A number of different drugs are currently in clinical use as H_2-receptor antagonists. Although considerable information is available with respect to their effects of gastric acid secretion, their comparative effects on the histamine response in airway smooth muscle have not been studied. Therefore, it is also unknown which H_2-receptor antagonist is the safest for asthmatic patients. In addition, particular H_2-receptor antagonists also might have other actions on airway smooth muscle beside a simple antagonism at H_2-receptors. Such other actions might include an agonist action at H_2-receptor or a direct stimulant or depressant effect on the tone of airway smooth muscle. The present investigation employed guinea pig tracheal smooth muscle to compare the effects of two new H_2-receptor antagonists (ranitidine and famotidine) with cimetidine and to assess both direct and receptor-mediated responses.

Methods

Male guinea pigs weighing 250–750 g were killed by a blow on the neck and the tracheas removed from larynx to carina. Two ring tracheal strips were prepared by modification of the technique previously reported,[11] and four preparations were obtained from each animal. Each preparation was mounted in an organ bath filled with 20 ml of Krebs-Ringer type solution maintained at 37° C and aerated with 5% CO_2 and 95% oxygen. The solution contained the following chemicals (mM): NaCl, 120.7; KCl, 5.9; $CaCl_2$, 2.5; $MgCl_2$, 1.2; $NaHCO_3$, 15.5; NaH_2PO_4, 1.2; and glucose, 11.5.

The isometric tension of each sample was continuously measured with a strain-gauge transducer (Minebea Co., Ltd., Japan) and recorded on pen oscillograph (San-ei instrument Co., Ltd., Japan). Resting tension of each sample was set at approximately 1.5 *g* before drug addition. The effects of H_2-receptor antagonists were tested on tracheal smooth muscle in its resting state and also on muscle that was contracted with histamine (3×10^{-7}, 3×10^{-6}, 10^{-5}, and 3×10^{-5} M) or bethanechol (10^{-5} and 10^{-4} M). The dose–response relationships for histamine (3×10^{-8}–3

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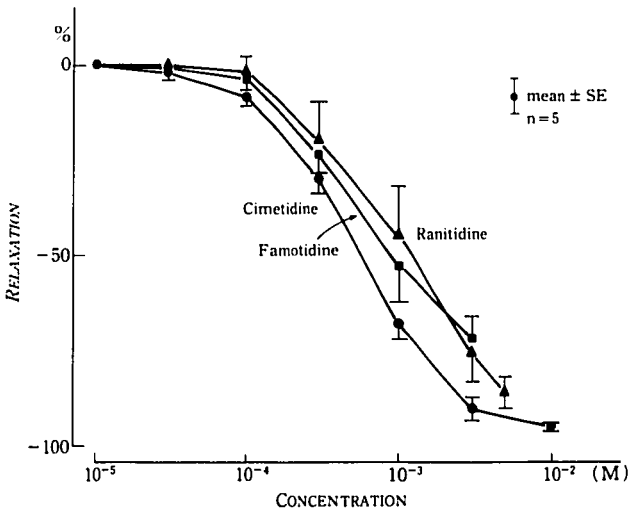

FIG. 1. Dose-related relaxation produced by H_2-receptor antagonists. 100% indicates the maximal relaxant effect of isoproterenol.

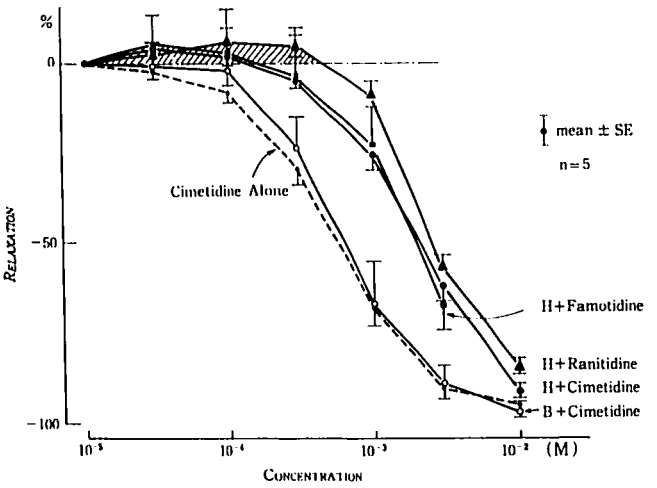

FIG. 2. Inhibition of relaxant effects of H_2-receptor antagonists in preparations pretreated with histamine 10^{-5} M (H) but not in preparations pretreated with bethanechol 10^{-5} M (B). The *hatched area* above 0% relaxation indicates further contraction of the histamine-pretreated muscles on addition of H_2-receptor antagonists. Antagonist concentrations of 10^{-5} M and below did not alter muscle tone.

$\times 10^{-4}$ M) and bethanechol (3×10^{-8}–3×10^{-4} M) also were examined in the presence and absence of cimetidine (cimetidine 10^{-6}, 10^{-5}, and 10^{-4} M for histamine curve, and 10^{-5} and 10^{-4} M for bethanechol study). Agonists were added to the organ baths in cumulative concentrations, and sufficient time was allowed to obtain the maximal effect of each concentration. Spontaneous tension change was also checked as a primary experiment. The tension change within 30 min was estimated to be less than 1% of maximum relaxation obtained from isoproterenol. Drug responses were compared with the maximum relaxation achieved by l-isoproterenol (10^{-6} M) at the end of each experiment, and every response was represented as a relative percentage of isoproterenol. Because it is easy to understand the degree of pharmacologic affinity, pD_2 values were obtained as the negative logarithm of the ED_{50} for relaxation or contraction. Differences in pD_2 were compared by the Student's *t* test for two groups or analysis of variance for more than two groups; $P < 0.05$ was considered significant.

Drugs were dissolved in 0.9% NaCl solution and were added to the organ baths in increments of 0.2 ml or less.

TABLE 1. pD_2 values* for Relaxation Elicited by H_2-Receptor Antagonists

H_2-Receptor Antagonists	No Pretreatment	Bethanechol (10^{-5} M) Pretreatment	Histamine (10^{-5} M) Pretreatment
Cimetidine	3.20 ± 0.04	3.15 ± 0.14	2.64 ± 0.04†
Ranitidine	2.95 ± 0.16	2.90 ± 0.11	2.53 ± 0.03‡
Famotidine	2.97 ± 0.14	3.00 ± 0.08	2.65 ± 0.15

pD_2 = negative logarithm of ED_{50}.
* Mean ± SE, n = 5.
† $P < 0.05$, ‡ $P < 0.001$, compared with no pretreatment.

Drugs used in this study were l-isoproterenol hydrochloride (Nikken), histamine dihydrochloride (Wako), bethanechol hydrochloride (Sigma), cimetidine hydrochloride (Fujisawa), ranitidine hydrochloride (Glaxo-Sankyo), famotidine hydrochloride (Yamanouchi), and propranolol hydrochloride (Sumitomo).

Results

When tested in uncontracted preparations, the H_2-receptor antagonists caused dose-related relaxation of resting tone (fig. 1) without evidence of an initial stimulatory effect. There were no significant differences between drugs in the mean pD_2 values for relaxation of resting tone (table 1). The dose–response curves for H_2-receptor antagonists were not altered by pretreatment with 10^{-6} or 10^{-5} M of propranolol, indicating a direct action on smooth muscle.

When muscles were precontracted with histamine 10^{-5} M (fig. 2), the H_2-receptor antagonists required about three-fold higher doses to elicit the same relaxant effect as in uncontracted preparations. There were no significant differences between drugs with respect to mean pD_2 values for relaxation of histamine-induced contraction (table 1). However, the pD_2 values were significantly less in histamine-pretreated preparations than in uncontracted preparations for both cimetidine ($P < 0.001$) and ranitidine ($P < 0.05$); the difference in pD_2 values for famotidine did not reach statistical significance (table 1).

When the muscles were precontracted with 10^{-5} M bethanechol, the pD_2 values for the relaxant effect of H_2-receptor antagonists were not different from those in un-

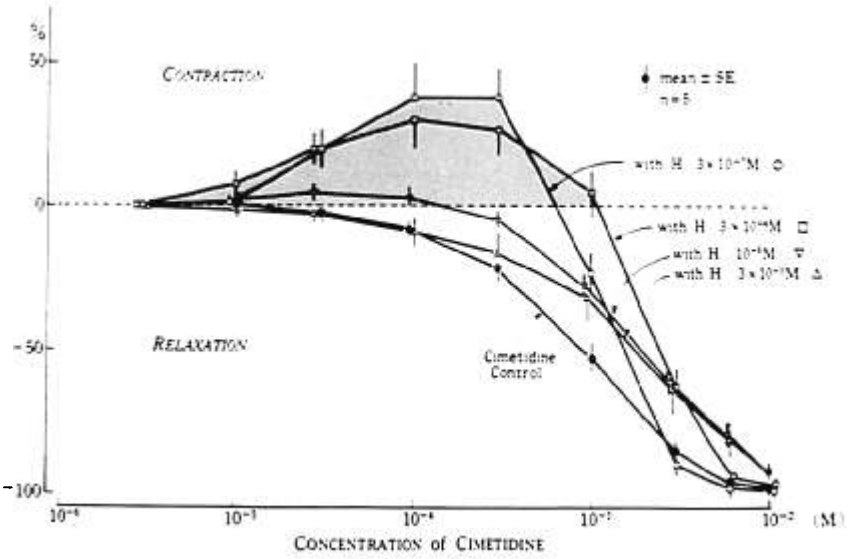

FIG. 3. Contractile and relaxant effects of cimetidine in preparations pretreated with various concentrations of histamine. The *dotted line* indicates muscle tone prior to addition of cimetidine. The *shaded area* above the line indicates contraction. Intensities of both contraction and relaxation (vertical axis) are expressed in terms of per cent of the maximal change in tension elicited by isoproterenol.

contracted preparations (table 1). The lack of effect of bethanechol on direct relaxant effect of H_2-receptor antagonists was not the result of a less intense test contraction, because at 10^{-5} M concentration, both bethanechol and histamine elicited contractions of approximately equal intensity.

Histamine- and bethanechol-pretreated muscles also differed with respect to their response to low concentrations of H_2-receptor antagonists. In the histamine-pretreated muscles, all three H_2-receptor antagonists elicited further contractions (potentiated the histamine contraction) at lower concentrations than needed to produce relaxation (fig. 2). The extent of contraction was quite similar for all H_2-receptor antagonists (fig. 2). On the other hand, contractile responses in bethanechol-pretreated muscles were not present following addition of cimetidine even in low concentrations, and cimetidine produced only a relaxant effect (fig. 2). Similar results were also observed in bethanechol-pretreated muscles following use of ranitidine or famotidine.

The effect of histamine concentration on the response to H_2-receptor antagonists was studied in further experiments using cimetidine (fig. 3). The pD_2 values for cimetidine-induced relaxation were significantly greater in uncontracted muscles than in those contracted with histamine; however, there were no significant differences in cimetidine pD_2 among the four different concentrations of histamine used as pretreatment (table 2). In contrast, the histamine concentration was of major importance in determining the extent of contraction elicited by low concentrations of cimetidine (fig. 3). Preparations that were strongly contracted with a high concentration (10^{-5} M) of histamine showed relatively slight contractile responses on addition of cimetidine, whereas the contracted responses to cimetidine were pronounced when tested in preparations that were only slightly contracted by a low concentration (3×10^{-7} or 3×10^{-6} M) of histamine (fig. 3). Moreover, ranitidine as well as famotidine produced considerable contraction at their low concentrations when pretreated with low concentration of histamine (3×10^{-7}, 3×10^{-6}, and 10^{-5} M). The contraction elicited by 3×10^{-5} M histamine was close to the maximal effect of this agonist. When tested against preparations that were pretreated with 3×10^{-5} M histamine, cimetidine produced little or no contraction (fig. 3), although the muscles were capable of considerable further contraction if exposed to high concentrations of bethanechol, such as 10^{-4} and 3×10^{-4} M.

In separate experiments, the effects of cimetidine pretreatment were tested on the subsequent dose–response curves for histamine and bethanechol. The dose–response curves for histamine were significantly shifted to the left by the pretreatment with various concentrations of cimetidine (10^{-6}, 10^{-5} and 10^{-4} M) (fig. 4). Values of pD_2 for histamine-induced contraction were increased (potentiation) by an approximately equal amount by pretreatment with cimetidine in concentrations ranging from 10^{-6} to 10^{-4} M (table 3). Cimetidine pretreatment also increased the maximal contractile response to histamine,

TABLE 2. Effect of Precontraction with Histamine on pD_2 Values for Relaxation Elicited by Cimetidine

Pretreatment	Cimetidine pD_2*	Significance
Control	3.05 ± 0.04	—
Histamine 3×10^{-7} M	2.82 ± 0.04	$P < 0.01$
Histamine 3×10^{-6} M	2.66 ± 0.06	$P < 0.001$
Histamine 1×10^{-5} M	2.72 ± 0.04	$P < 0.001$
Histamine 3×10^{-5} M	2.75 ± 0.09	$P < 0.05$

* Mean ± SE, n = 5.

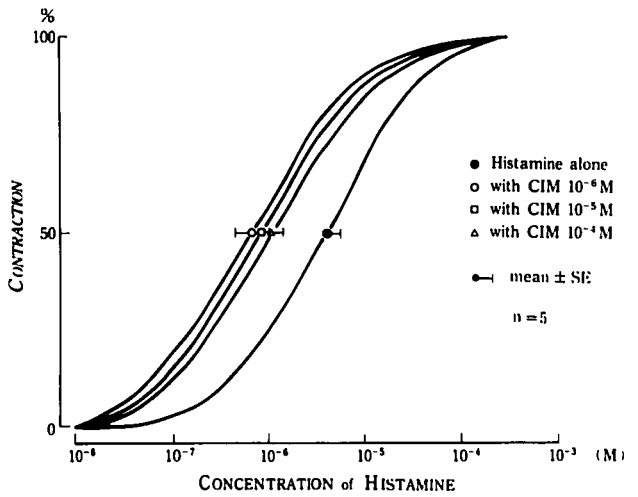

FIG. 4. Dose-related contraction produced by histamine. 100% indicates the maximal contractile response to histamine itself. The dose–response curves for histamine pretreated with cimetidine (CIM 10^{-6}, 10^{-5}, and 10^{-4} M) significantly shifted to the left (potentiation).

which was 17 ± 4, 19 ± 3, and 25 ± 3 (±SE) per cent greater than the control maximal response following pretreatment with cimetidine 10^{-6}, 10^{-5}, and 10^{-4} M, respectively. Values of pD_2 for bethanechol-induced contraction were not influenced by pretreatment with 10^{-5} or 10^{-4} M cimetidine (table 4), nor did cimetidine alter the maximal contractile response to bethanechol. The same results (potentiation of histamine-induced contraction but not in that of bethanechol-induced contraction) were also found when tested in ranitidine and famotidine pretreatments.

Discussion

All H_2-receptor antagonists produced dose-related relaxation of both resting tone and agonist-induced contractions. Such relaxation resulted from a direct effect on smooth muscle rather than release of catecholamines from nerve terminals, since it was not blocked by propranolol. These relaxant effects, *per se*, are of little clinical significance because they required concentrations far in excess of those achieved during clinical use of H_2-receptor antagonists. For instance, the plasma concentrations of ranitidine in patients taking therapeutic doses have ranged

TABLE 3. Effect of Pretreatment with Cimetidine on pD_2 Values for Contraction Elicited by Histamine

Pretreatment	Histamine pD_2*	Significance
Control	5.37 ± 0.11	—
Cimetidein 10^{-6} M	6.20 ± 0.17	$P < 0.01$
Cimetidine 10^{-5} M	6.11 ± 0.15	$P < 0.01$
Cimetidine 10^{-4} M	6.08 ± 0.21	$P < 0.05$

* Mean ± SE, n = 5.

TABLE 4. Effect of Pretreatment with Cimetidine on pD_2 Values for Contraction Elicited by Bethanechol

Pretreatment	Bethanechol pD_2*	Significance
Control	5.35 ± 0.10	—
Cimetidine 10^{-5} M	5.55 ± 0.09	NS
Cimetidine 10^{-4} M	5.36 ± 0.04	NS

* Mean ± SE, n = 5.
NS = not significant.

from 10^{-7} to 10^{-6} M,[12] several orders of magnitude less than threshold concentrations *in vitro* for ranitidine-induced relaxation. The *in vitro* relaxant effects, however, do demonstrate a greater effectiveness (table 1) against resting tone or bethanechol-induced tone than against histamine-induced tone. Similarly, over a wide range of concentrations (10^{-6}–10^{-4} M), cimetidine potentiated the response to histamine (table 3) but had no effect on that to bethanechol (table 4). These differences in cimetidine against histamine as opposed to bethanechol, spare or potentiate the histamine-induced contraction and demonstrate a substantial relaxant effect of histamine acting at H_2-receptors, which in part antagonized the stimulant effect of histamine at H_1-receptors. Potentiation of the histamine-induced contraction was evident at much lower concentrations of H_2-receptor antagonists than required to elicit direct relaxation of resting tone (fig. 2) and were particularly evident in experiments involving pretreatment with histamine before the addition of cimetidine (fig. 3). Potentiation of the histamine-induced contraction was also emphasized by pretreatment with various concentrations of cimetidine. This was evident by the significant shift of histamine dose–response curve to the left (fig. 4 and table 3). The further contraction occurring on addition of cimetidine was most pronounced in muscles that were not strongly contracted by histamine. In this situation, even 10^{-5} M cimetidine produced a noticeable contraction or augmentation of the histamine response (fig. 3). This unmasked contraction was represented not only in cimetidine but also ranitidine and famotidine, although the data are not shown. Therefore, the relative magnitude of the contraction revealed at low concentration of H_2-receptor antagonists, especially when pretreated with low concentration of histamine (3×10^{-7} and 3×10^{-6} M), may be clinically meaningful. Such contractions did not represent a direct effect of cimetidine on smooth muscle, an agonist effect at H_1-receptors, or release of endogenous histamine because in the absence of histamine pretreatment, the H_2-receptor antagonists did not increase airway tone (fig. 1). Our study, therefore, provides further evidence for the presence of H_2-receptors in guinea pig tracheal smooth muscle and for a modulating role of such receptors on bronchoconstrictor responses to histamine.

Our data *in vitro* may not necessarily be extrapolated to humans because of the relative balance of H_1- and H_2-receptors. However, because cimetidine achieves concentrations ranging from 10^{-6} to 10^{-4} M during clinical therapy,[13] the concentration range associated with potentiation of histamine-induced contraction in our experiments overlaps that achieved in clinical use. On the other hand, augmentation of histamine-induced contraction was not observed at a concentration below 10^{-5} M for any of the three H_2-receptor antagonists. Thus, although there are substantial differences in the potency of H_2-receptor antagonists in reducing gastric acid secretion[14,15] and equivalent differences in clinical doses, the concentrations required to augment histamine-induced contraction in our studies were about the same ($\geq 10^{-5}$ M). If the potencies of histamine-induced contraction are similar among H_2-receptor antagonists, large-dose requirement of H_2-receptor antagonists should have a greater effect on the constriction of the airway. Therefore, we estimate that the likelihood of producing airway constriction during treatment with H_2-receptor antagonists will be greatest for those requiring high clinical doses and least for those administered in low dose: cimetidine > ranitidine > famotidine.

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